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THE ADRENAL CORTEX AND ADMINISTRATION OF ACTH, CORTISONE AND A COMBINATION OF ACTH AND CORTISONE

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INTRODUCTION

As early as a couple of decades ago it was observed that cortical adrenal extracts induced atrophy of the suprarenal gland in laboratory animals (Ingle & Kendall 1937, Wells & Kendall 1940). By the use of pure glucocorticoids, this phenomenon could subsequently be verified in numerous experimental investigations. That cortisone therapy brings about atrophy of the suprarenal gland also in human beings has often been noted in autopsies performed on patients treated with the drug (e.g. O'Donnel & al. 1951, Fraser & al. 1952, Solassa & al. 1953, Bennet 1953, Stoner & Whiteley 1954). Bennet's series comprised 190 patients who had received cortisone before death. If the amount of cortisone acetate exceeded 450 mg, the weight of the adrenals decreased and the sudanophilia diminished — in such a way, however, that if treatment had been terminated more than six weeks before death, changes could not be detected. A reversible phenomenon is thus involved. Solassa & al. could no longer detect atrophy if treatment had been discontinued > 10 days before death. Clinically it could also be ascertained that adrenal steroid production was more or less impaired up to ten days after the discontinuation of cortisone therapy (e.g. Forsham & al. 1950, Wilkins & al. 1951). Even during long-term cortisone therapy, however, it has been possible to provoke endogenic steroid production in the aldrenals by means of ACTH (e.g. Fischer & Hansen 1955, Hansen & al. 1955).

ACTH induces hypertrophy of the suprarenal gland in both laboratory animals and humans, with the zona fasciculata, in particular, becoming enlarged as much as twofold. A diminishing of the sudanophil materials and specifically the birefrigent lipoids, as an indication of augmented secretory activity, is

another typical change met with in the suprarenal gland during ACTH therapy (e.g. Landing & Feriozi 1954, Stoner & Whiteley 1954, Eränkö 1955, Klärner 1955, Wexel & Rinfret 1955, Symington 1956). According to Klärner the weight of the suprarenal gland increases upon prolonging of ACTH dosages, but only up to the third month; starting with the fourth month, the hyperplasia begins to retrogress, the weight decreases nearly to normal and fats begin to become stored up in the cells.

The changes induced by exogenic cortisone and ACTH in the suprarenal gland are nearly opposite. The condition following cortisone treatment is liable to be dangerous to the human being, for adaptability to external stress has decreased; thus, for instance, such a patient's resistance under a surgical operation without cortisone protection decreases and the consequence is liable to be severe shocks or death (e.g. Fraser & al. 1952, Solassa & al. 1953).

Winter & al. (1953) were able to prevent adrenal atrophy produced by cortisone in laboratory animals with androgens. Recently both experimental and clinical observations have been presented that, by administering ACTH simultaneously or occasionally during cortisone treatment, atrophy of the suprarenal body otherwise developing during this treatment could be prevented (Rawls & al. 1954, Wolfson 1954, Birke & al. 1956 Christy & al. 1956).

The purpose of this study is expressly to note the changes occuring in the cortex of the suprarenal body of rats after combined ACTH + cortisone treatment, as compared to the changes taking place after cortisone or ACTH treatment.

PRESENT STUDY

MATERIAL AND METHODS

Male rats were used as test animals, so that the periodic variations in the adrenals effected by the oestrus would not hamper the evaluation of the results. The animals weighed 226 — 317 g.

The ACTH group comprised 20 animals, each of which for a period of seven days received daily three i.u. Cortrophin-Z (Organon Oss), an ACTH preparation. The cortisone group comprises 23 animals, each of which for a period of seven days received 5 mg injections of cortisone acetate. Sixteen animals received daily three i.u. ACTH and 5 mg cortisone acetate. In all the groups the injections were given subcutaneously in the back. The test animals were killed with a blow on the head in the morning between 9 and 10 o'clock before being fed. The killings were done 1, 4, 7, 15, 21 and 30 days after discontinuation of treatment; on the same days fourteen animals who had undergone no treatment were also killed. Immediately after the death of the animals, both suprarenal bodies were removed and weighed in a torsio balance; then the weight was recorded per 100 g of the body weight, using the mean weight of both glands. For the Sudan staining (Sudan III), one of the suprarenal glands was fixed for 24 hours in neutralized 4 % formol. Morphological examination of the glands took place after a 5-hour fixing in Zenker's solution and after staining with haematoxylin-eosin.

RESULTS

Weight of the Suprarenal Body. — The weights of the suprarenal body are presented in Fig. 1.

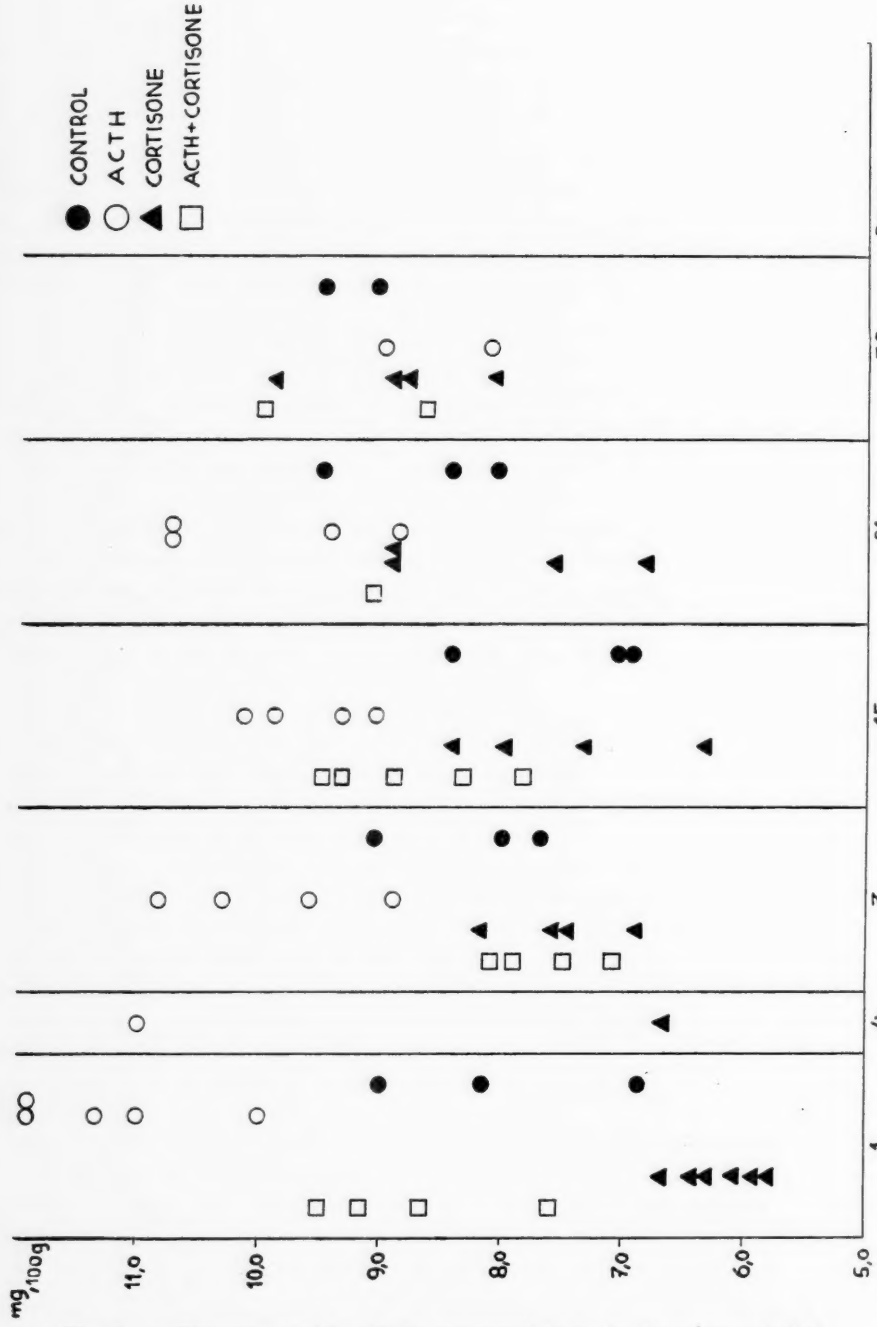

Fig. 1. — The mean weight of the suprarenal body in the groups studied, at different times, after administration of cortisone, ACTH or a combination of cortisone and ACTH, as compared to the weight of the gland of control animals killed about the same time.

Fig. 1 shows how after administration of ACTH the weights have clearly increased; in the cortisone group the weights remain below normal, whereas in animals that have received both ACTH and cortisone the weights correspond to those of the control animals. A week following the termination of treatment the suprarenal gland of those that had received cortisone begins to regain normal weight; but in the ACTH group it remains above normal. As much as two weeks after discontinuation of treatment in the ACTH group, the weight remains above normal, whereas in the cortisone group it has already become restored to normal. The situation after three weeks and after 30 days may be regarded as normal in respect to weights. Throughout the entire investigation phase the weight of the suprarenal body of the animals in the group receiving combined ACTH + cortisone treatment remains within the limits of the controls.

To sum up, our series suggested that the drug-induced specific changes in the weight of the suprarenal gland of the rat can be prevented by simultaneous administration of ACTH and cortisone.

MICROSCOPICAL FINDINGS

Controls

Haematoxylin-eosin Staining: The cells of zona glomerulosa generally have vacuoles of varying size. The transitional zone is to be observed in all the animals on account of its denser cytoplasm and the fact that the nuclei are situated close together. In the outer parts of zona fasciculata the cells are usually vacuolated. The size of the vacuoles varies considerably. In the inner parts of this zone the vacuoles nevertheless prove to be of the same size, or relatively small. The cells of the zona reticularis are quite eosinophilic with respect to their plasma and in only certain cells are small vacuoles to be seen.

Sudan Staining. — The glomerular zone generally contains a moderate amount of sudanophil drops, which are on the whole small of size. The zone is generally stained uniformly, but sometimes spotty staining is met with. In all the control animals

there was to be observed a sudanophobic zone (= transitional zone in haematoxylin-eosin staining). The outer parts of the fascicular zone contained appreciably more sudanophil drops than the inner parts. The size of the drops varies from animal to animal. Zona reticularis is generally very weakly sudanophil, with lipoid drops being noted only here and there.

Groups Treated

Findings on the first day after discontinuation of treatment:

ACTH group: Zonation is seen in Plate 1. Deviating from normal is the fact that no transitional zone whatsoever occurs. The entire cortex is thicker than normal and this thickening has taken place mainly in the fascicular and reticular zones.

The cells of the glomerulosa (Plate 12) have more than the normal quantity of secretion drops; very few of the sinusoids are open. The fascicular cells (Plate 23) are supplied with an unusually rich eosinophilic plasma. There is a relative paucity of secretion drops. A scantiness of secretion drops is to be observed (Plate 27) in the reticular cells, and the sinusoids are enlarged.

Sudanophilia is most clearly evident in the glomerulosa. The sudanophobic zone normally occurring between the glomerulosa and the fasciculata is missing altogether. The fascicular and reticular zones are very slightly sudanophil (Plate 33).

Cortisone group: Zonation is presented in Plate 8. It is to be observed that the transitional zone comes into view plainly and more broadly than normal. The zona fasciculata and reticularis are thinner than normal.

The glomerular cells (Plate 19) hardly differ from normal. Less than the normal number of sinusoids are open. The cells of the clear and abnormally broad transitional zone are markedly eosinophilic and densely packed. The fascicular cells (Plate 25) are exceedingly hypertrophic and contain an abundance of large secretion drops. The reticular cells (Plate 31) are densely packed and filled with small secretion drops. Very few of the sinusoids are open.

Sudanophilia is most clearly evident and equally marked in the outer part of the glomerulosa and fasciculata. Stainig is regular and moderately strong. A sudanophobic zone considerably broader than normal is to be observed between the glomerulosa and the fasciculata (Plate 35).

ACTH + Cortisone Group: The zonation is shown in Plate 5. The transitional zone is clearly discernible. The zona fasciculata is slightly thinner than normal and the reticularis, again, slightly thicker.

In the glomerular cells (Plate 16) are to be seen more than the normal number of secretion drops and its outermost cells are small; their plasma is markely eosinophilic, while further within the plasma is paler and the vacuoles are smaller. The cells of the transitional zone are eosinophilic; the layer does not come into view continuously but only in places. The fascicular cells do not appreciably deviate from normal. In the reticularis (Plate 29) the cells are of ordinary size, their plasma is densely eosinophil and the sinusoids are enlarged.

The glomerulosa is rather weakly but evenly sudanophil. A distinct sudanophobic zone is to be perceived. The outer part of the fasciculata is a more distinctly sudanophil area; on the other hand, the sudanophilia in its inner parts is weak. The reticular area has a pale red color, which becomes stronger at the boundary between the cortex and the medulla, where the size of the sudanophil drops increases (Plate 34).

Comments on the findings of the first day: In respect to zonation, the ACTH + cortisone group comes nearest to being normal. In the ACTH group, the sudanophobic (= transitional) zone is lacking. The fascicular cells contain less secretion drops than normal, whereas in the cortisone group they contain more than normal. This would indicate that the secretion had not been discharged by the cells during cortisone treatment, although the formation of intracellular secretion had taken place. The ACTH + cortisone group is most nearly normal in this respect as well. In the zona reticularis, attention is attracted by the abundance of sinusoids in the ACTH and ACTH + cortisone groups as well as their remarkable paucity in the cortisone group. This is probably to be interpreted in such a way that, on account of excessive exogenic cortisone production, the transfer of endo-

genic secretion products into the blood stream has diminished. With respect to sudanophilia, attention is attracted not only by the afore-mentioned absence of the sudanophobic zone in the ACTH group, its abnormally prominent occurrence in the cortisone group and its most nearly normal occurrence in the ACTH + cortisone group but also by the fact that in the ACTH group the most distinct sudanophil zone is the glomerulosa, in the cortisone group the glomerulosa and the outer part of the fasciculata and in the ACTH + cortisone group the outer part of the fasciculata.

Findings on the fourth day after discontinuation of treatment:

ACTH group: Zonation shown in Plate 2. No transitional zone can yet be demonstrated. The fasciculata and reticularis are still higher than normal.

The plasma of the glomerular cells (Plate 13) has become appreciably more eosinophilic and the number of secretion drops diminished; on the other hand, sinusoids are met with in considerable abundance. The fascicular cells (Plate 24) are now throughout the whole layer exceedingly hypertrophic and full of secretion drops. Also in the reticular cells there now occur more secretion drops.

Sudanophilia is now most marked in the fasciculata. No sudanophobic zone can yet be demonstrated.

Cortisone group: Zonation is shown in Plate 9. The transitional zone has become narrower. The fasciculata and reticularis have begun to thicken.

The quantity of secretion drops in the fascicular cells (Plate 20) has quite clearly decreased in comparison with the situation observed on the first day (Plate 26). More sinusoids are beginning to appear in the reticularis.

With respect to sudanophilia, changes have taken place only in the outer parts of the fasciculata, where the size of the sudanophilic drops has diminished in comparison with the results of the first day.

ACTH + cortisone group: Lacking.

Comments on the findings of the fourth day: In the ACTH group no transitional zone is yet to be observed; in the cortisone group it has narrowed. An interesting observation is the inverse relation of the secretion drops of the fascicular cells in the

ACTH and cortisone groups as compared with the findings for the first day. The explanation most likely lies in the fact that, in the ACTH group, the extra exogenic stimulation of the cortex of the suprarenal body, has not been present, in consequence of which the cells discharge the secretion at normal speed. In the cortisone group, normal ACTH secretion of the hypophysis begins to operate and the secretion collected in the cells is thereby discharged.

Findings on the seventh day after discontinuation of treatment:

ACTH group: Zonation shown in Plate 3. The transitional zone is hard to define. The glomerulosa is lower than normal. The fasciculata has slightly narrowed, compared with the fourth day; the reticularis is still higher than normal.

In glomerular cells (Plate 14), secretion drops have continued to diminish in quantity, and the abundant sinusoids noted on the fourth day are now less open. In fascicular cells the quantity of secretion drops has decreased in comparison with the fourth day; they continue to occur in greater than normal abundance, as Plate 14 shows. In reticularis attention is attracted by the enlarged sinusoids (Plate 28).

Now a distinct sudanophobic zone is to be observed — which was not yet evident on the fourth day. Sudanophilia continues to be most distinct in the zona fasciculata, which is uniformly red; its cells contain relatively large lipoid drops. The reticularis is practically sudan-negative.

Cortisone group: With respect to zonation, the situation begins to be normal; the transitional zone is difficult to distinguish (Plate 10).

Of the glomerular cells (Plate 21), part are poor in plasma, and part include large droplets, which press the nucleus flat. In the fascicular cells, nothing deviating from normal can be indicated. In the reticular cells (Plate 32), extremely small secretion grains and abundant open sinusoids can be seen.

Sudanophilia in the glomerulosa has become uneven. The sudanophobic zone has narrowed from the preceding.

ACTH + cortisone group: With respect to zonation (Plate 6), attention is attracted by the fact that the fasciculata is still lower and the reticularis higher than normal. The transitional zone becomes visible (most clearly in Plate 17).

The glomerular cells (Plate 17) are all poor in plasma and no secretion drops can be detected in them, as on the first day. The sinusoids of the layer are larger than usual. The fascicular cells do not deviate from normal. In the reticularis the sinusoids continue to be larger than normal and its cells contain tiny secretion drops (Plate 30).

In the glomerulosa is to be perceived a uniform sudanophilia, and a distinct sudanophobic zone exists. The fasciculata is uniformly and markedly sudanophil; in the reticularis the sudanophilia remains as before.

Comments on the findings of the seventh day: With respect to zonation, the situation is reverting to normal. Attention is drawn to the fact that in the ACTH group there has now appeared a sudanophobic zone. The abnormally large quantity of secretion drops noted on the first day in the glomerular cells of the ACTH and ACTH + cortisone groups is beginning to revert to normal. In general, moreover, the situation appeas, on microscopic examination to approach normal on the part of all the groups.

Microscopically examined, the findings on the fifteenth, twenty-first and thirtieth days after discontinuation of treatment are most nearly normal. The situation of the fifteenth day is presented in Plates 4, 7, 11, 15, 18 and 22.

DISCUSSION

The most distinct differences between the various groups were naturally apparent immediately after the discontinuation of treatment. Then the zona fasciculata and zona reticularis were higher than normal in the ACTH group, and lower than normal in the cortisone group, while in the ACTH + cortisone group the zona fasciculata had become lower but the reticularis resembled most closely the ACTH group. In the group thus combined, the contrary changes evidently bring it about that no changes in the weight of the suprarenal body occur. An interesting fact is that the transitional (= sudanophobic) zone becomes visible in both the cortisone and ACTH + cortisone groups — whereas it appears in the ACTH group only after a week, and then, too,

at first most clearly only as a sudanophobic zone. Although the zona fasciculata was atrophic in the cortisone group, its cells were nevertheless hypertrophic and contained an abundance of secretion drops. Our explanation for this was that the cortisone, by inhibiting endogenic ACTH production, had prevented a discharge of the secrete of the fascicular cells but not the secretion itself. This findings would well explain how, even during a long and strong exogenic cortisone suppression, it is possible by means of ACTH to induce glucocorticoid secretion. Cain & Harrison (1950) have pointed out that in the fascicular cells of a rat it is possible to distinguish a secretory phase and a discharge phase. Our results would speak in favor of the exogenic cortisone suppression's most readily affecting expressly this discharge phase of the fascicular cells by inhibiting it. It would seem logical to think that the transitional zone is actually one which, according to need, produces fasciculata. When the discharge phase of the fascicular cells has been inhibited by the addition of exogenic cortisone, the transition of the cells between the fasciculata and the glomerulosa is as if dammed, which would lead to enhanced occurrence of the transitional zone in the cortisone group. In the ACTH group the discharge phase of the fascicular cells is enhanced through the addition of exogenic stimulation, and the transition of the cells between fasciculata and glomerulosa is so rapid that a transitional zone cannot form. The absence of this zone in the ACTH group could thus be explained. The fact that in the ACTH + cortisone group this zone remains fairly normal rather than becoming accentuated, as in the cortisone group, would indicate that even during exogenic cortisone suppression it is possible with ACTH to stimulate physiologically not only the discharge phase of the fascicular cells but also the transition of cells occurring between the glomerulosa and the fasciculata. By means of the doses used in the present investigation, this addition of exogenic ACTH during cortisone suppression has not, however, been able to prevent atrophy of the fasciculata; but it has, on the other hand, sufficed to induce the changes in the glomerulosa and reticularis that are quite comparable to the group receiving ACTH treatment alone. This would indicate that the strongest effect of exogenic cortisone suppression and the one most difficult to

inhibit with ACTH operates on the fasciculata. Since changes are brought about by ACTH in all layers of the cortex, of which exogenic cortisone is capable of clearly weakening only the phenomena occurring in the fasciculata, our results would make evident that the glucocorticoids are only a certain group of substances whose secretion is regulated by ACTH in the suprarenal cortex and that the discharge of these glucocorticoids from the cells takes place in the fasciculata.

Our results would indicate that at least a periodic use of cortisone and ACTH in combination is clinically defensible, if the condition of the suprarenal gland is considered. As for the effect of the combination on the hypophysis and its possible atrophy, it is a matter apart and would require clarification.

SUMMARY

The authors have studied the effect of the administration of ACTH, cortisone and ACTH + cortisone on the suprarenal glands of rats. The series consisted of 73 male rats, of which 14 were controls. Hormones were administered for a week. Following treatment, the animals were killed immediately and after 4, 7, 15 and 30 days. Attention was directed in the suprarenal body to the weight, morphology and sudanophilia.

The weight of the suprarenal body in the ACTH group had risen immediately following the discontinuation of treatment; in the cortisone group it was low and in the combined group normal. After 15 days the weights in the ACTH and cortisone groups had likewise by and large become normal.

In the ACTH group, the sudanophobic zone was lacking on the first and fourth days following the discontinuation of treatment, but it reappeared on the seventh day. In the cortisone group, this zone was accentuated; and in the ACTH + cortisone group, it was normal.

In the ACTH group, the zona fasciculata and reticularis were hypertrophic, while in the cortisone group, they were atrophic. In the ACTH + cortisone group, the fasciculata was slightly atrophic and the reticularis hypertrophic. After 15 days, the zonation situation had become normal in every group.

In the cortisone group, a considerable quantity of secretion drops was met with in the fascicular cells after discontinuation of treatment; as early as the fourth day, the drops had been secreted by the cells. On the basis of this and other findings, the present authors submit that exogenic cortisone suppression specifically prevents the discharge phase of the fascicular cells.

The present authors believe that the clinical use of ACTH and cortisone combination — at least intermittently — has a beneficial effect on the suprarenal body. How such therapy might affect the hypophysis remains an open question.

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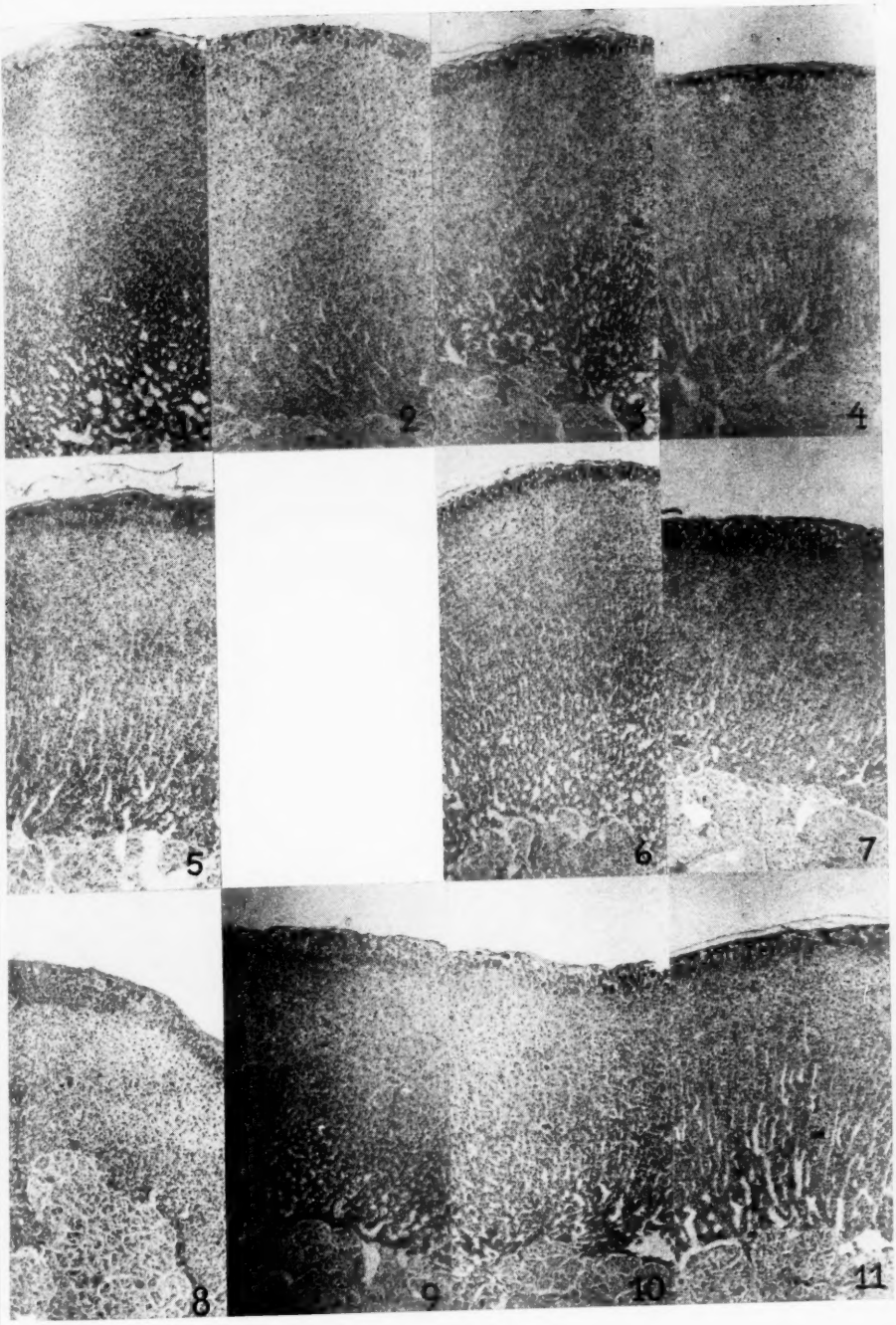
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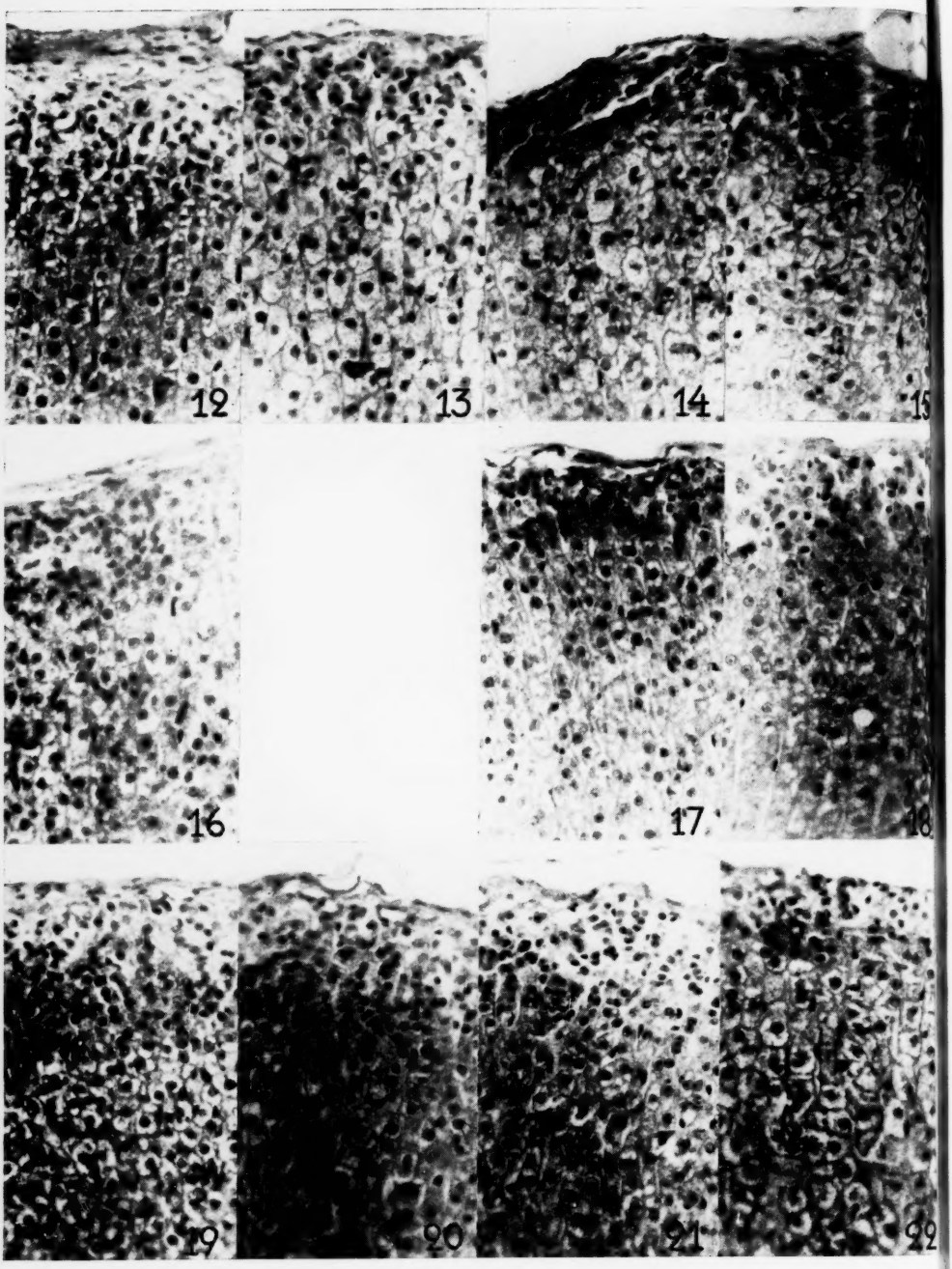
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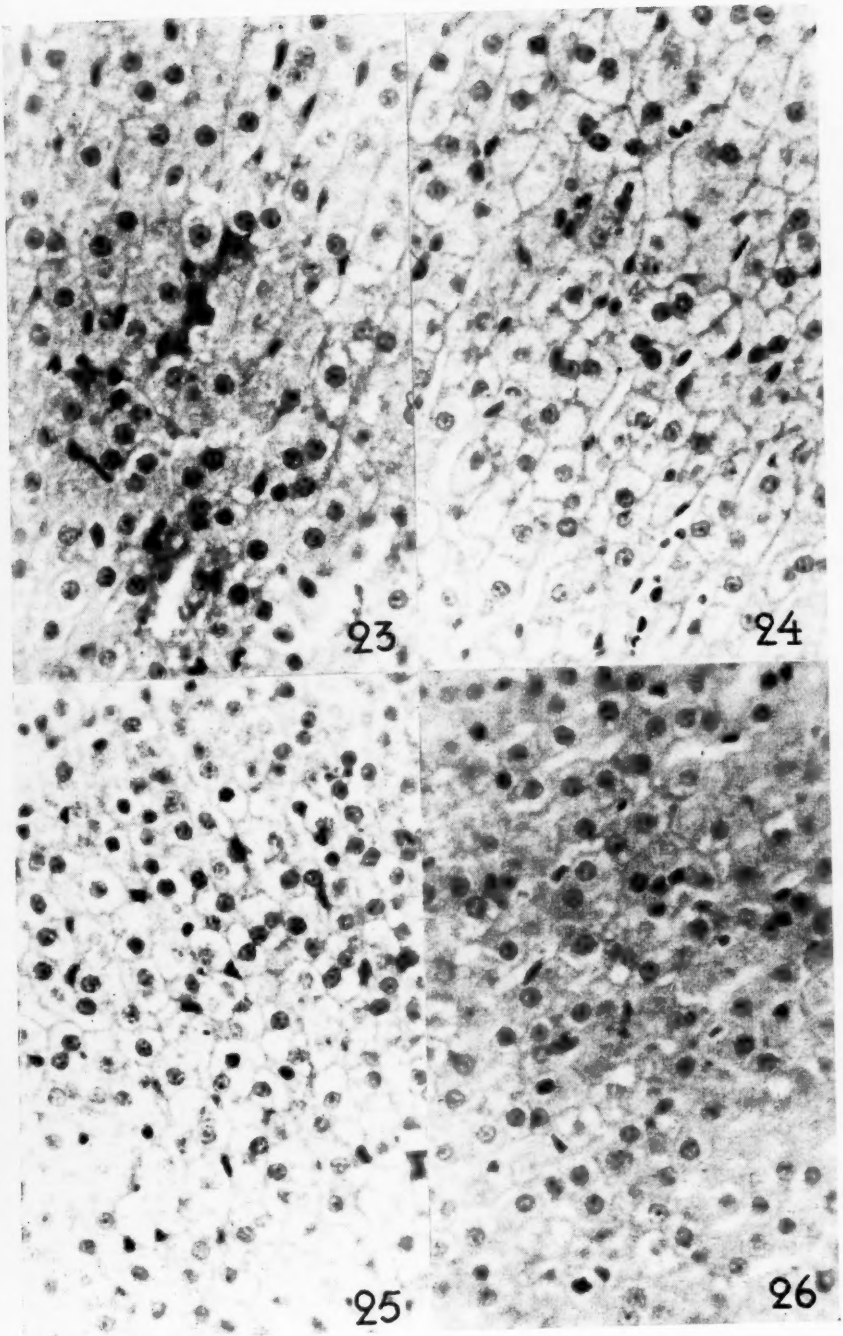
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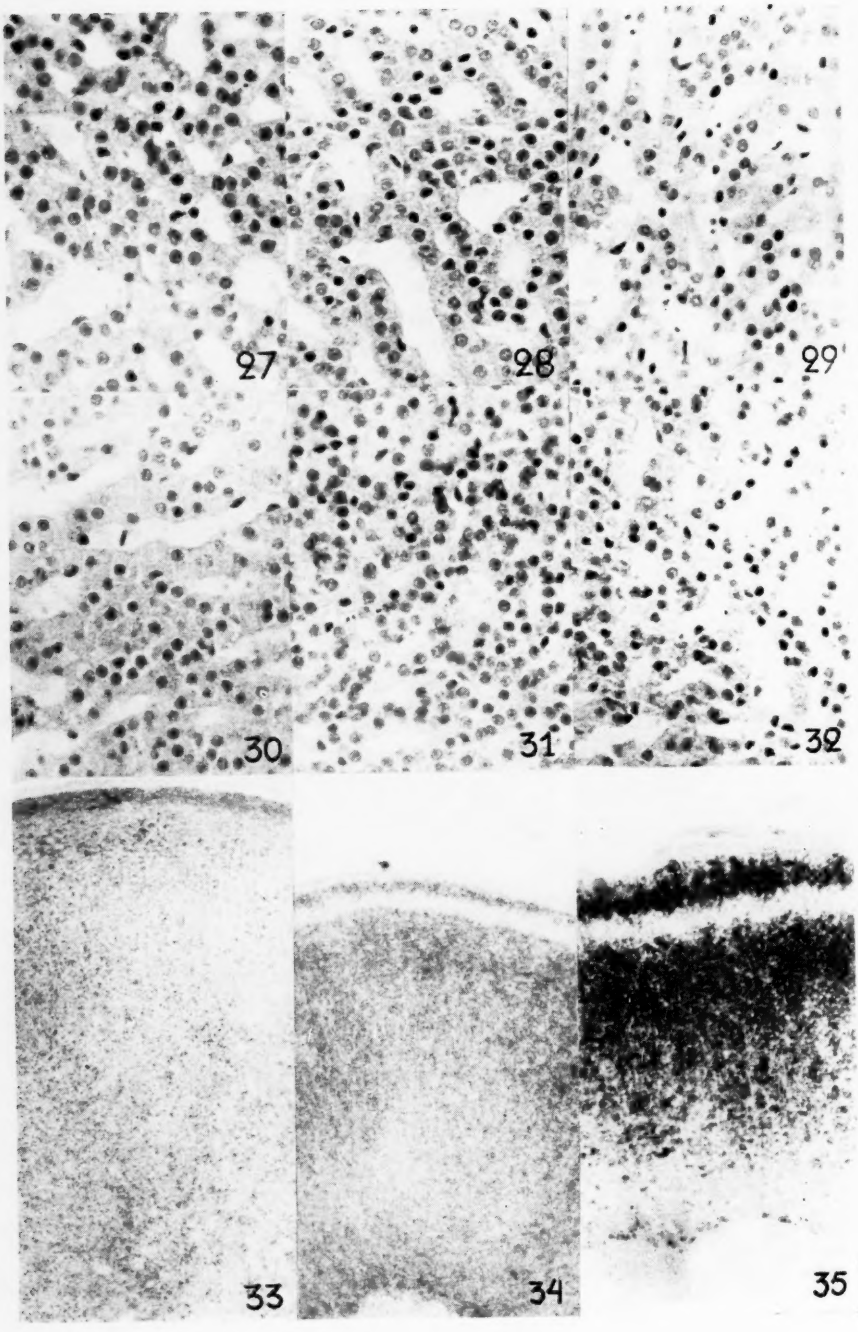
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